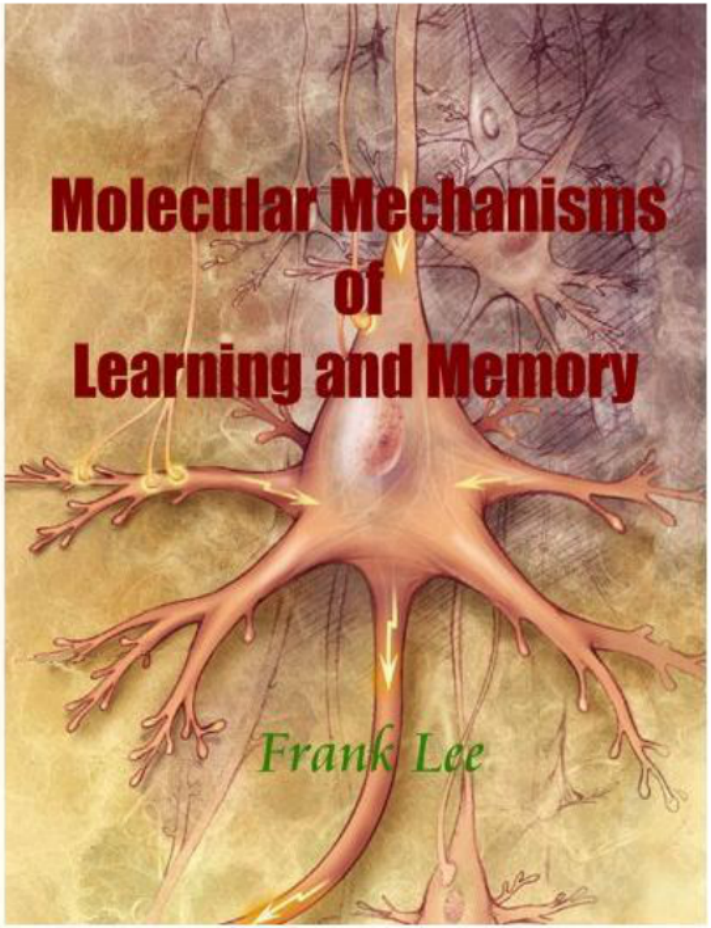
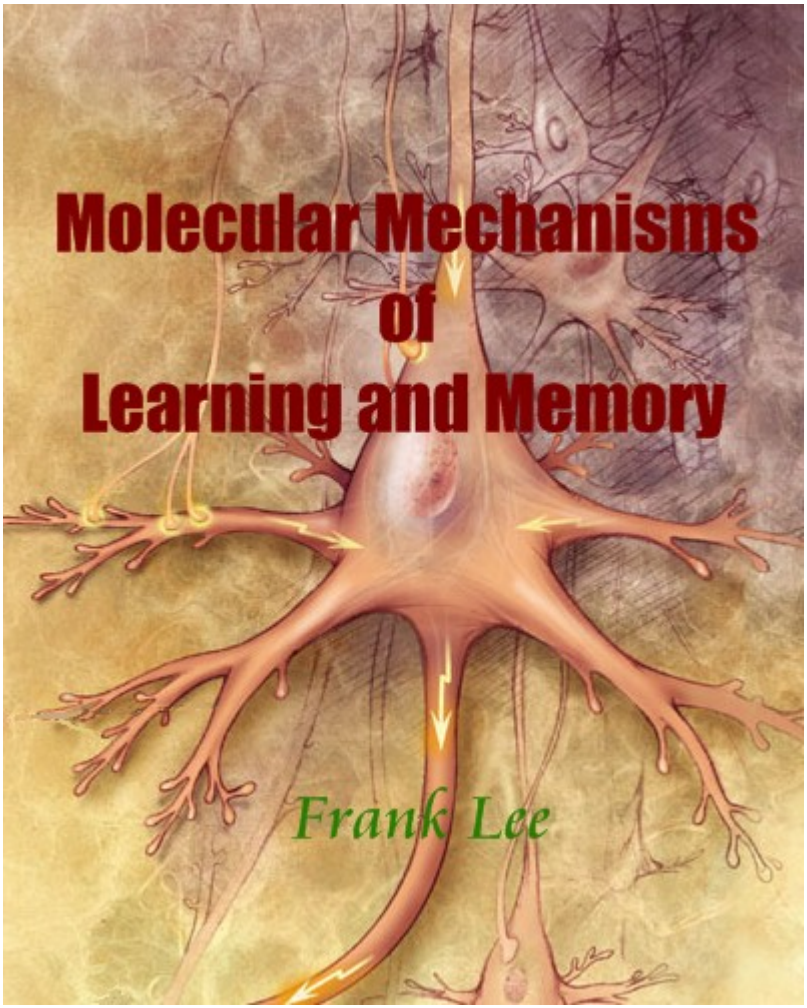

Molecular Mechanisms
of
Learning and Memory
Frank Lee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1. Introduction

Albert Einstein said, "The most incomprehensible fact about nature is that it is comprehensible." Indeed, physical phenomena are comprehensible. Weather changes, chemical reactions and molecular structures appear quite complex, but their underlying principles are amazingly simple. All physical phenomena are governed by four fundamental forces - gravity, electromagnetism, strong and weak nuclear forces. Recently, these forces can further be unified by a single theory -- the string theory.

What about the brain? Is its ability of comprehension comprehensible? In the seventeenth century, Isaac Newton had discovered the gravitational law and Robert Boyle had formulated the equation for gas properties, but human beings were still quite ignorant about physiological systems. William Harvey, a pioneer in blood circulation, told Robert Boyle that it was the valves in the veins which guided him to the next phase of his thinking.

We now know that thoughts are not controlled by blood vessels and sorrow has nothing to do with a "broken heart". They come from our brain -- the only thing which can try to understand itself. With a size smaller than a microwave oven, our brain contains more than 10 billions of neurons. Each neuron is connected to many (up to thousands) other neurons. The junction between neurons is called a **synapse**. Brain functions are the results of signal transmission through trillions of synapses.

Since neurons are made up of the same atoms as found in the physical world, it might be possible to explain brain functions in terms of physical laws. In the past few decades, significant progress has been made toward this goal. The formation of short-term memory through learning has been well understood. However, conversion from short-term to long-term memory is still a mystery. In Chapters 9 and 10, we shall present a possible mechanism which involves **microtubules**.

Nervous systems, like other communication systems, use a sequence of impulses to carry message. The nature of nerve impulses, however, differs entirely from electromagnetic waves and sound waves. In every nerve cell, there is a membrane separating the cytoplasmic fluid from the extracellular solution. The transmembrane voltage (also called **membrane potential**) is defined as the inside potential minus the extracellular potential. When the nerve fiber is at rest, the membrane potential is about -70 mV. The nerve impulse is a sharp change of the membrane potential. Therefore, it is also known as the **action potential** (Fig. 2.1).

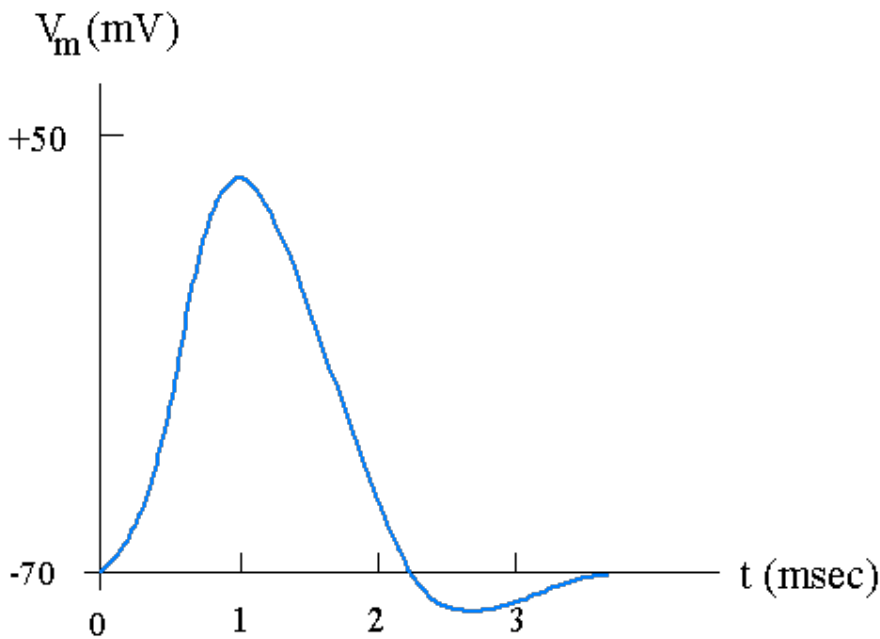

Figure 2.1. A typical nerve impulse (action potential).

Ion Conductances

The generation of action potentials is mainly due to the changes of sodium (Na^+) and potassium (K^+) conductances. Figure 2.2 shows the concentrations of Na^+ and K^+ ions on both sides of a nerve membrane. For Na^+, its concentration on the extracellular side is

much higher than inside. We immediately notice that Na^+ ions are far from electrochemical equilibrium -- both the electric force due to electric potential difference and the chemical force due to ion concentration difference are pointing inward. How could the nerve membrane maintain such a stable state? This is because the conductance of Na^+ ions in the membrane is very small at the resting membrane potential. Although the inward driving force is large, the resulting Na^+ influx is small. This small influx can be balanced by a slow ion transport process, the Na^+-K^+ pump, which moves Na^+ ions outward and simultaneously K^+ ions inward.

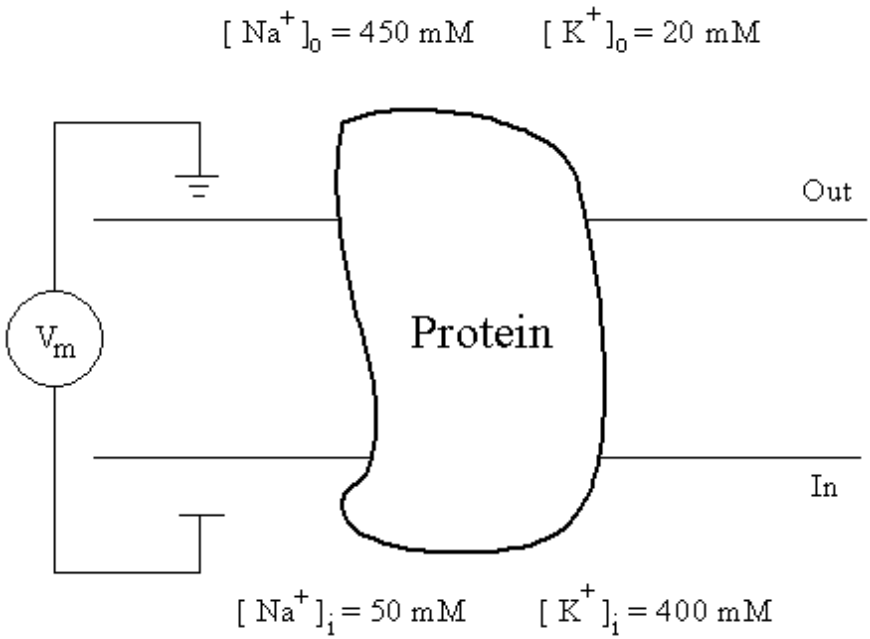

Figure 2.2. Ion concentrations on both sides of a nerve membrane, which contains various types of proteins. Ions may move across the membrane through a special class of proteins called ion channels.

The conductance of Na^+ ions may change dramatically with the membrane potential as demonstrated by voltage clamp experiments, in which the membrane potential is displaced to a new value and maintained there (Fig. 2.3). Because ions carry charges, the movement of ions across the membrane will change the membrane potential. To maintain a constant membrane potential, the voltage clamp circuit must generate electric currents to neutralize the membrane potential

change caused by the ionic flux. Thus, the ion current through the membrane is reflected in the electric current of the voltage clamp circuit outside the membrane.

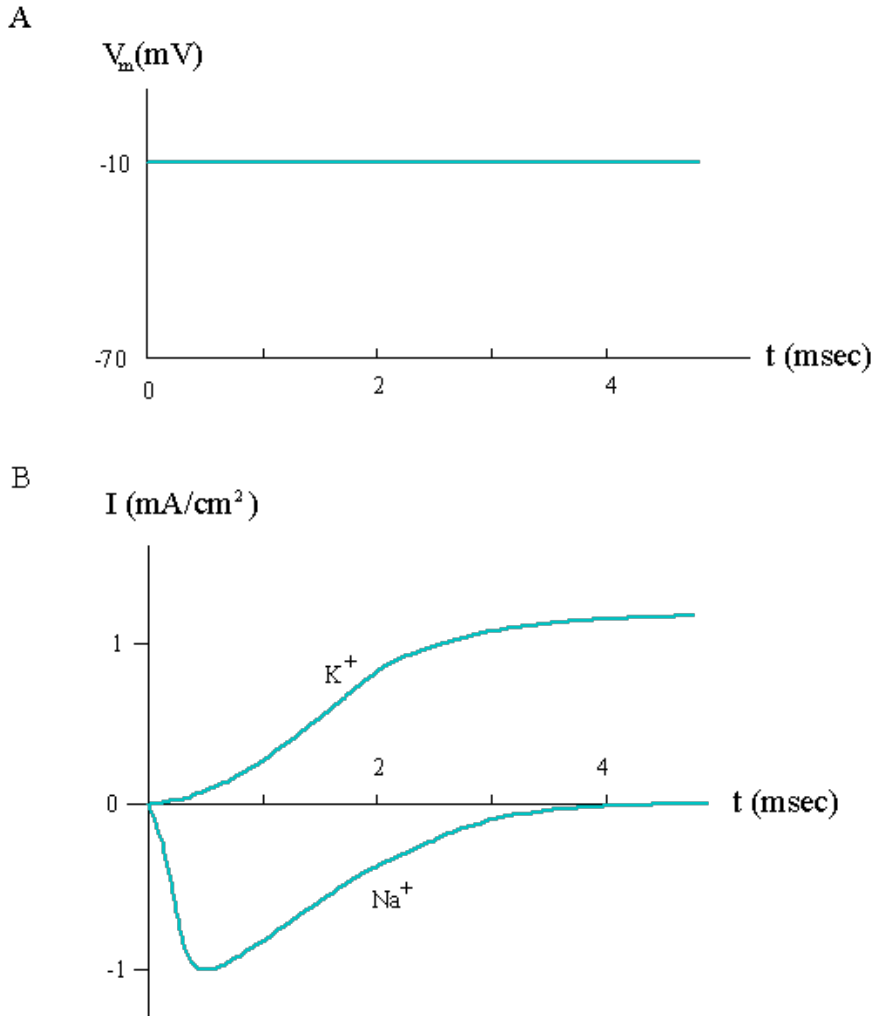

Figure 2.3. Ion currents measured from the voltage clamp circuit. (A) The membrane potential is depolarized from -70 mV to -10 mV at t = 0, and kept constant by the voltage clamp circuit. (B) The resulting Na^+ and K^+ currents through the membrane (outward positive) as measured from the voltage clamp circuit.

A membrane is said to be **depolarized** if the new potential V_m is more positive than the resting potential V_{m0}, and **hyperpolarized** if

$V_m < V_{m0}$. Since depolarization makes the inside potential more positive, we expect (from the consideration of electrochemical forces) the currents of all cations to increase in the outward direction. Fig. 2.3 shows the experimental result for squid axons. The K^+ current indeed increases in the outward direction. However, the behavior of Na^+ current is totally unexpected. It first rises sharply in the inward direction and then declines with a slower rate to its resting value. Phenomenologically, the change of the Na^+ current can be attributed to the change in Na^+ conductance. Experimental results indicate that the Na^+ conductance increases in the early stage of depolarization, which exceeds the reduction in the inward driving force. As time proceeds, the Na^+ conductance decreases, resulting in the late decline of Na^+ currents. The rise and fall of Na^+ conductance are known as **sodium activation** and **sodium inactivation**, respectively. They play important roles in the generation of a nerve "impulse", which must contain a rising phase as well as a falling phase. Mathematically, the ion conductance is defined by

$$G_{ion} = I_{ion}/(V_m - V_{ion}) \qquad (1)$$

where I_{ion} denotes the ion current through the membrane (outward current is defined as positive), and V_{ion} is the equilibrium potential for the ion. According to the Nernst equation,

$$V_{ion} = (RT/F) \ln ([ion]_o/[ion]_i) \qquad (2)$$

where R denotes the gas constant, T is the absolute temperature, F represents the Faraday constant, $[ion]_o$ and $[ion]_i$ are the ion concentrations in the extracellular and intracellular fluids, respectively.

At room temperature, the equilibrium potential for Na^+ with concentrations given in Fig. 2.2 is +55 mV. When the membrane potential is depolarized, I_{ion} varies with time as shown in Fig. 2.3. From eq.(1), we can obtain the time course of the ion conductance. Fig. 2.4 shows the experimental results for a few depolarizations. We see that the peak of Na^+ conductance increases with increasing depolarizations before it levels off at large depolarizations. Another feature to remember is that the rising rate of G_K is much slower than G_{Na}.

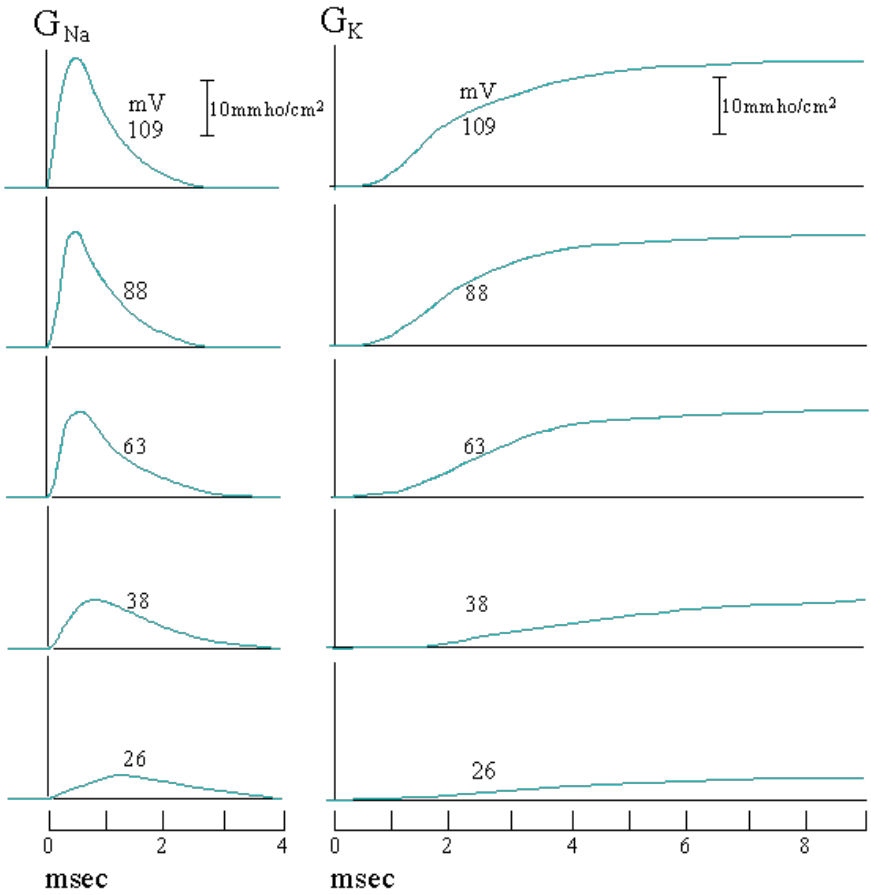

Figure 2.4. Time courses of Na^+ and K^+ conductances at a few depolarizations. The number next to each curve represents the depolarizing voltage from the resting potential -70 mV.

The ion conductance change is the collective results of many individual ion channels. Each ion channel may be in either "open" or "closed" state. The opening probability of each ion channel depends on the membrane potential, but the detailed mechanism is not known.

The Generation of action potentials

In excitable cells, the action potential is elicited when the membrane potential is depolarized to a critical value (the threshold). In most axons, the threshold is about 15 mV above the resting potential. Before the threshold is reached, the axon membrane

behaves like an ordinary inactive substance with a certain resistance and capacitance. When a constant current I_s is applied to the inactive membrane, the membrane potential becomes,

$$V_m(t) = I_s R_m[1 - \exp(-t/R_m C_m)] + V_{m0} \qquad (3)$$

where R_m and C_m are the effective resistance and capacitance of the membrane, respectively.

As shown in Fig. 2.4, the peak of G_{Na} increases with increasing depolarizations. When the membrane is depolarized, more Na^+ ions will flow into the intracellular fluid. Because the Na^+ ions carry positive charges, the Na^+ influx will make the membrane more depolarized, which in turn makes the Na^+ conductance even larger. In the mean time, the K^+ conductance also increases with increasing depolarization. In the physiological range of membrane potentials and ion concentrations, the electrochemical force on K^+ is outward. The outflux of K^+ will make the membrane more hyperpolarized, counteracting the Na^+ influx. However, the rising rate of G_K is much slower than G_{Na}. In the early stage, the rise of K^+ outflux will not be as large as the Na^+ influx except at small depolarizations. Therefore, when the membrane is depolarized to a critical value, above which the Na^+ influx exceeds the K^+ outflux, the Na^+ ions will flow inward at an accelerating rate. As a result, the membrane potential rises sharply until it approaches the equilibrium potential for Na^+. Subsequently, the sodium inactivation sets in and the K^+ conductance rises significantly. The membrane potential then returns to its resting value, constituting the falling phase of the action potential.

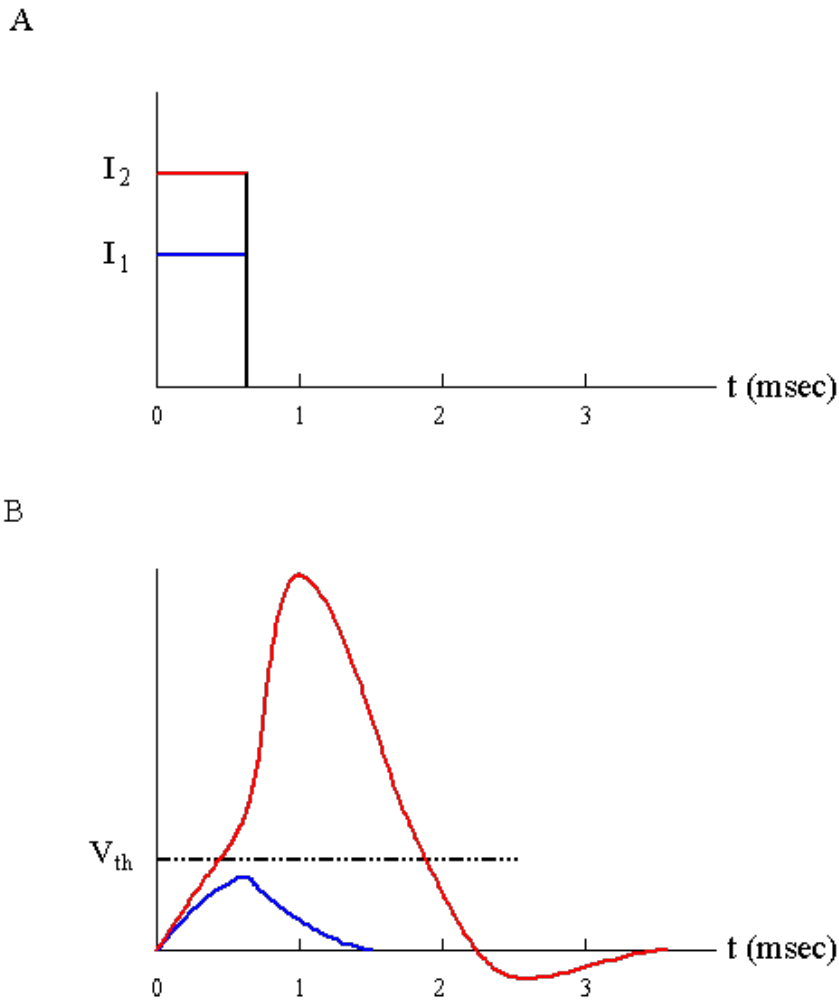

Figure 2.5. The behavior of all or none. (A) A current pulse, I_1 or I_2, is applied to a nerve membrane. (B) I_1 fails to increase the membrane potential to the threshold whereas I_2 is strong enough to elicit a nerve impulse.

If the stimulus fails to raise the membrane potential to the threshold, the intrinsic sharp potential change cannot occur. This behavior is known as "all or none". Figure 2.5 illustrates the membrane response to two different current pulses, I_1 and I_2. The magnitude of I_1 is too small to charge the membrane above the threshold, but I_2 is strong enough to initiate an action potential.

3. Ion Channels

Ion channels are a special class of proteins that conduct small ions such as Na^+, K^+, Ca^{2+} or Cl^-. The opening and closing of ion channels may depend on membrane voltage or the binding of molecules (the ligand). Its mechanism remains unclear. However, the mystery of their selectivity has been solved.

Ion Selectivity

Potassium Channels

By definition, a potassium channel conducts mainly K^+ ions while excluding Na^+ and Ca^{2+} ions. We note that a Na^+ ion is smaller than a K^+ ion. How could a channel conduct K^+ ions while excluding smaller ions? The answer lies in the ion's hydration energy.

The diameter of the potassium channel's **selectivity filter** (the narrowest region of its channel pore) should be about the same as the size of a Cs^+ ion (diameter = 3.3Å) because Cs^+ is the largest ion which can barely pass through the potassium channel. In a solution, ions are surrounded by water molecules. The first hydration shell of a K^+ or Na^+ ion contains six water molecules. The diameters of unhydrated Na^+ and K^+ ions are 1.96Å and 2.66Å, respectively. The effective diameter of a water molecule in a hydrated ion is larger than 2Å. Therefore, to pass through the selectivity filter, the K^+ or Na^+ ion must remove four water molecules from its first hydration shell, leaving only two (one at the front and anther at the back). The smaller Na^+ ion requires greater dehydration energy than the K^+ ion, because its nucleus has shorter distance with surrounding water molecules and thus interacting more strongly. As a result, the Na^+ ion is harder to pass through the potassium channel than the K^+ ion. The observation that lithium (Li^+) is excluded from the potassium channel can also be explained by the same energetic consideration. Divalent cations such as Ca^{2+} should also be excluded because their dehydration energies are much higher than monovalent cations.

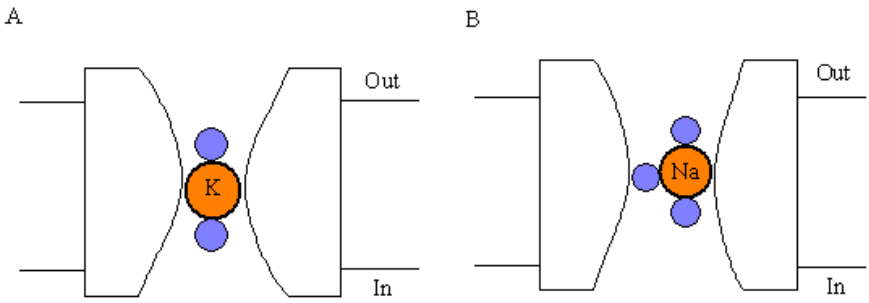

Figure 3.1. Ion passage through potassium and sodium channels. (A) To pass through the potassium channel, an ion must remove most of its surrounding water molecules, leaving only two - one at the front and another at the back. (B) The selectivity filter of the sodium channel is slightly larger than that of the potassium channel. It may accommodate a Na^+ ion attached with three water molecules, but not enough for a K^+ ion attached with three water molecules.

Sodium Channels

In the sodium channel, the Na^+ ion is more permeable than the K^+ ion. This is because the selectivity filter of the sodium channel is slightly larger than that of the potassium channel. It is large enough to accommodate a Na^+ ion attached with three water molecules, but not enough for a K^+ ion attached with three water molecules. Therefore, to pass through the sodium channel, the Na^+ ion needs to remove only three, but the K^+ ion has to remove four, water molecules from its first hydration shell. The required dehydration energy for the K^+ ion is greater than the Na^+ ion.

Calcium Channels

In calcium channels, the permeability of monovalent cations (Na^+ and K^+) is about three orders of magnitude smaller than the Ca^{2+} permeability. This ion selectivity does not seem to involve hydration, because Ca^{2+} is more heavily hydrated than Na^+, and the unhydrated diameters of Ca^{2+} and Na^+ are almost identical. Then, how could calcium channels select Ca^{2+} over Na^+?

Although the permeability of monovalent cations in the calcium channel is quite small at normal ionic concentrations, large monovalent cationic current can be observed in the absence of Ca^{2+}

and other divalent cations. This suggests that the calcium channel is basically permeable to both divalent and monovalent cations, but the selectivity arises from competition between ions. The calcium channel may contain a negatively charged binding site to facilitate ion conduction. The monovalent cations simply cannot compete with Ca^{2+} for this binding site. This idea has been confirmed experimentally. In the calcium channel, if a negatively charged glutamate residue in the pore-lining region is mutated into a positively charged lysine, the calcium channel becomes more permeable to Na^{+} than Ba^{2+} (reference). Conversely, in the sodium channel, mutation of a pore-lining lysine residue into glutamate transforms the channel from a Na^{+}-selective to a Ca^{2+}-selective channel (reference).

Channel Structures

K^{+} Channels

There are many types of potassium channels. The one involved in the generation of action potentials is composed of four subunits, each is homologous to the Shaker protein (Fig. 3.2). The hydrophobicity profile indicates that it contains six hydrophobic segments, designated as S1 - S6. These segments are likely to be the transmembrane domains. Other experimental results suggests that the P-region is lining the channel pore. The domain structure of the Shaker protein is shown in Fig. 3.3.

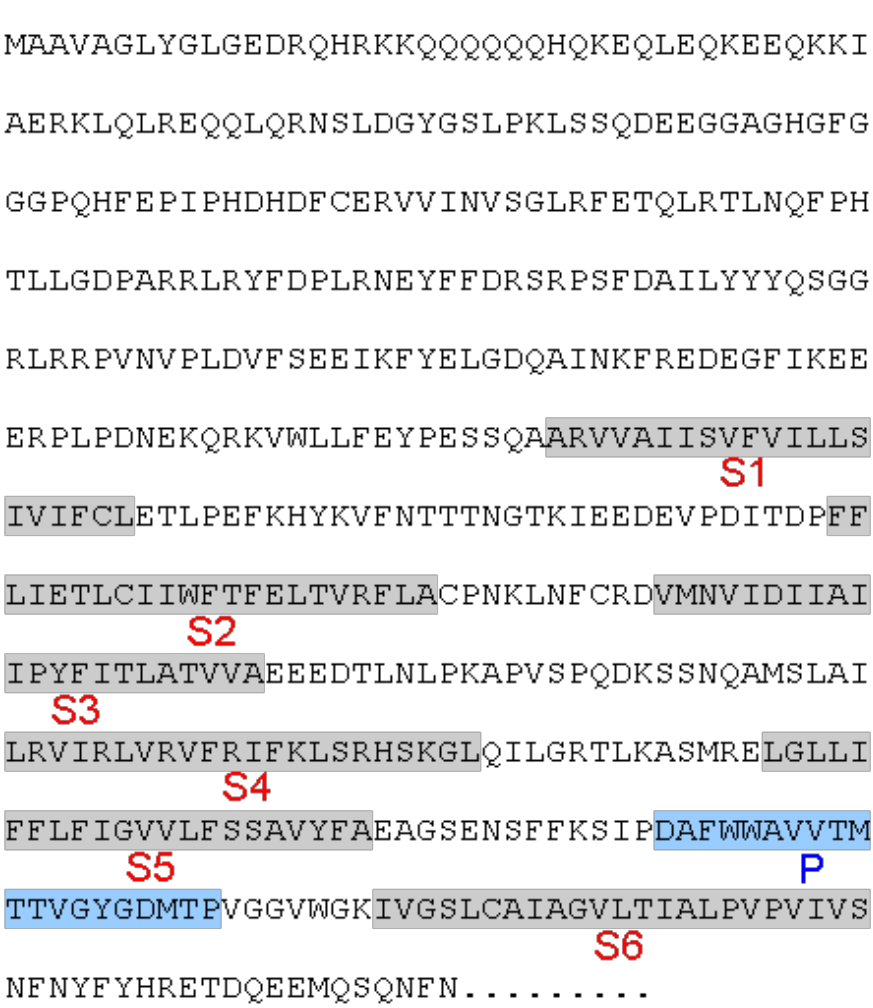

Figure 3.2. The amino acid sequence of the Shaker protein.

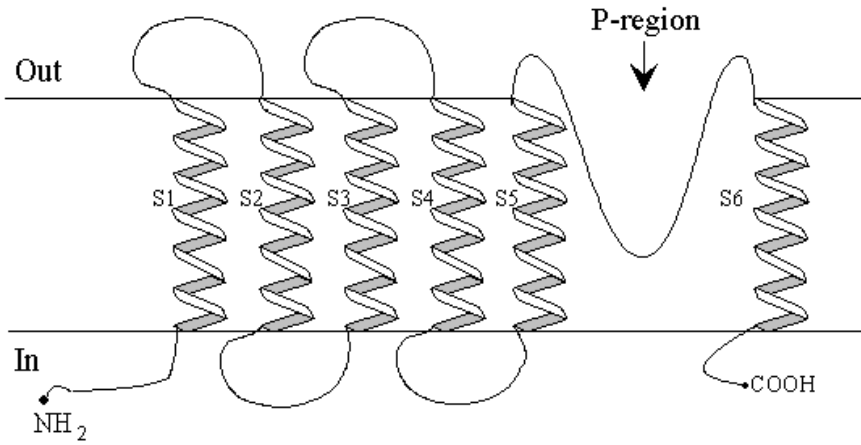

Figure 3.3. The domain structure of the Shaker protein.

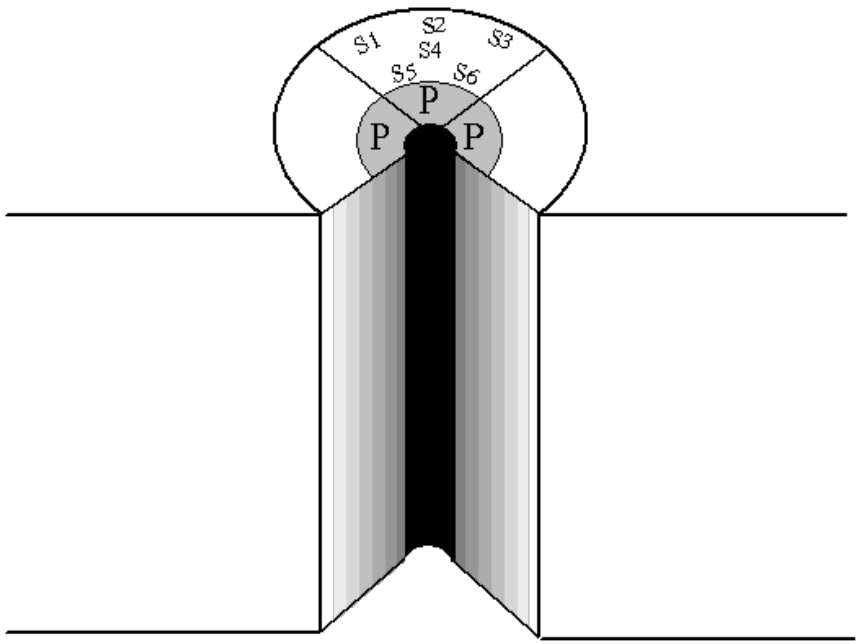

Figure 3.4. Schematic drawing of the Shaker potassium channel, which is composed of four Shaker proteins. The channel pore is formed by four P-regions (only three are shown here).

A growing number of channel structures have been determined by x-ray crystallography (web link). These structures were obtained in

the absence of membrane environment. Furthermore, an antibody Fab was used to stablize crystal structures. Whether or not the crystal strutures represent native structures remains a question (reference).

Na^+ and Ca^{2+} Channels

A Na^+ or Ca^{2+} channel consists of a major pore-forming subunit and possibly other small auxiliary subunits. The major pore-forming subunit is called the α subunit which can be divided into four similar domains. Each domain is analogous to a Shaker protein with six transmembrane segments and a P-region. Thus, an α subunit is sufficient to form an ion channel.

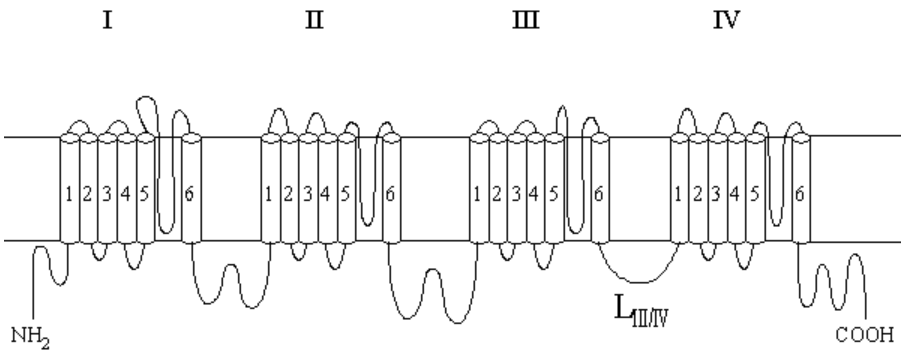

Figure 3.5. The domain structure of the α subunit of a Na^+ or Ca^{2+} channel.

Synaptic Channels

A large class of ion channels are specifically located at the synapse (the junction between neurons). Each synaptic channel consists of five subunits. Figure 3.6 shows the general structure of a nicotinic acetylcholine receptor (nAChR) channel.

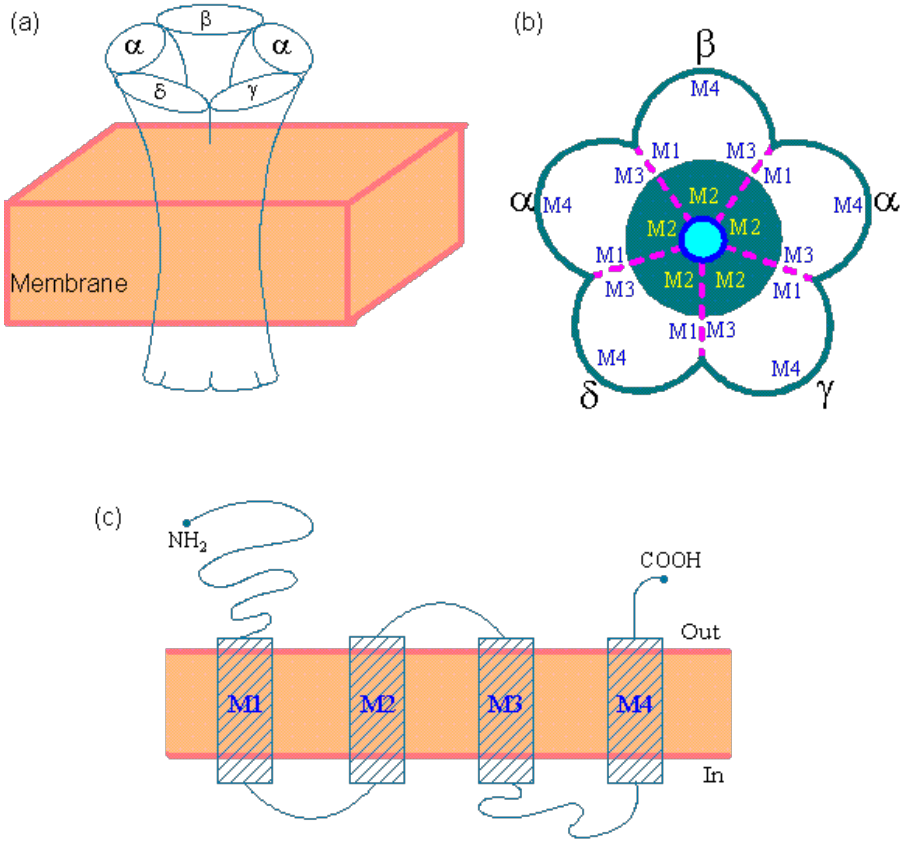

Figure 3.6. Schematic drawing of the nAChR channel which consists of five subunits: α, α, β, γ, and δ. **(a)** Side view. **(b)** Top view. **(c)** The domain structure for each subunit. Five M2 segments (one from each subunit) form the channel pore as shown in (b). Activation (opening) of an nAChR channel requires binding of two acetylcholine molecules, one on each α subunit.

4. Synaptic Transmission

A neuron is composed of three parts: cell body (soma), one or more dendrites, and a single axon. The cell body is similar to other types of cells, containing a nucleus and other typical organelles. The dendrites project from the cell body to receive signals from other neurons. The axon extends from the cell body to transmit the signals to other neurons through synapses. A synapse is a junction between an axon terminal and a dendrite, typically at **spine** (a small membranous protrusion from the dendrite). An axon may have one or more terminals and a dendrite may contain many spines. Thus, a single neuron can receive inputs from many neurons and transmit to many neurons.

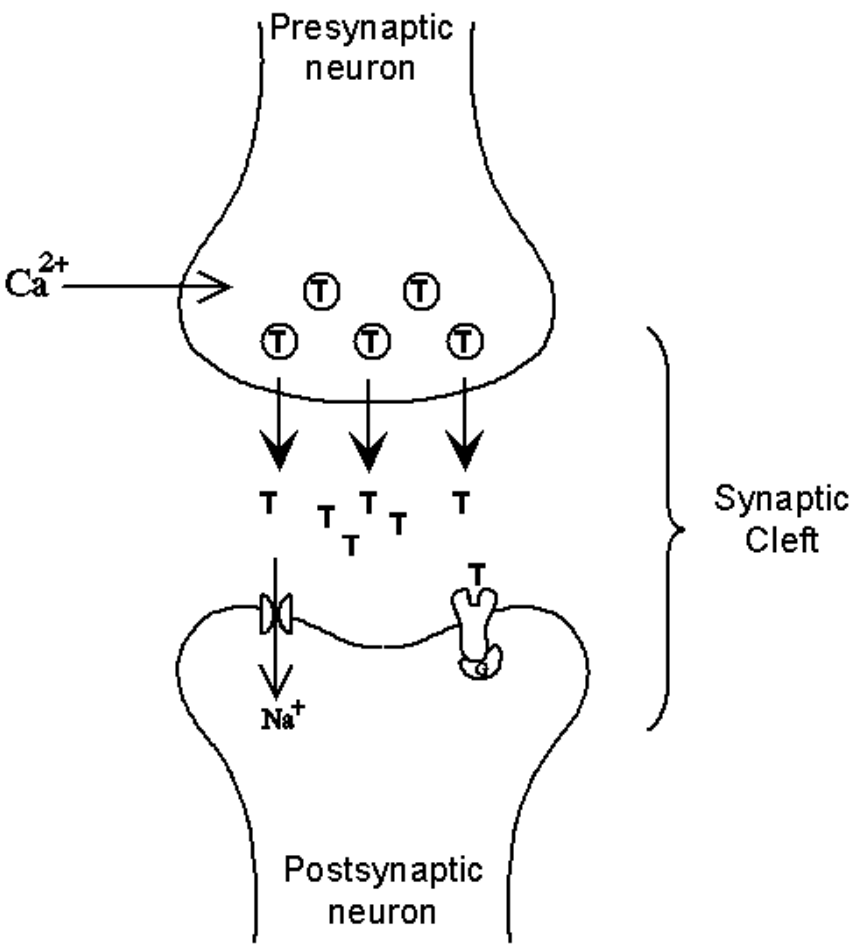

Figure 4.1. The mechanism of synaptic transmission. "T" represents the neurotransmitters which are stored in vesicles (represented by circles) at the presynaptic axon terminal. The action potential at the presynaptic terminal causes the entry of Ca^{2+} ions through voltage-activated calcium channels, leading to the release of neurotransmitters.

At the axon terminal, neurotransmitters are stored in vesicles. When the nerve impulse arrives, membrane depolarization will open calcium channels for the entry of Ca^{2+} ions, which then induce the release of neurotransmitters stored in the vesicles. The neurotransmitters then diffuse through the synaptic cleft (about 200 - 500 Å wide) to act on their receptors in the dendrite of a postsynaptic neuron.

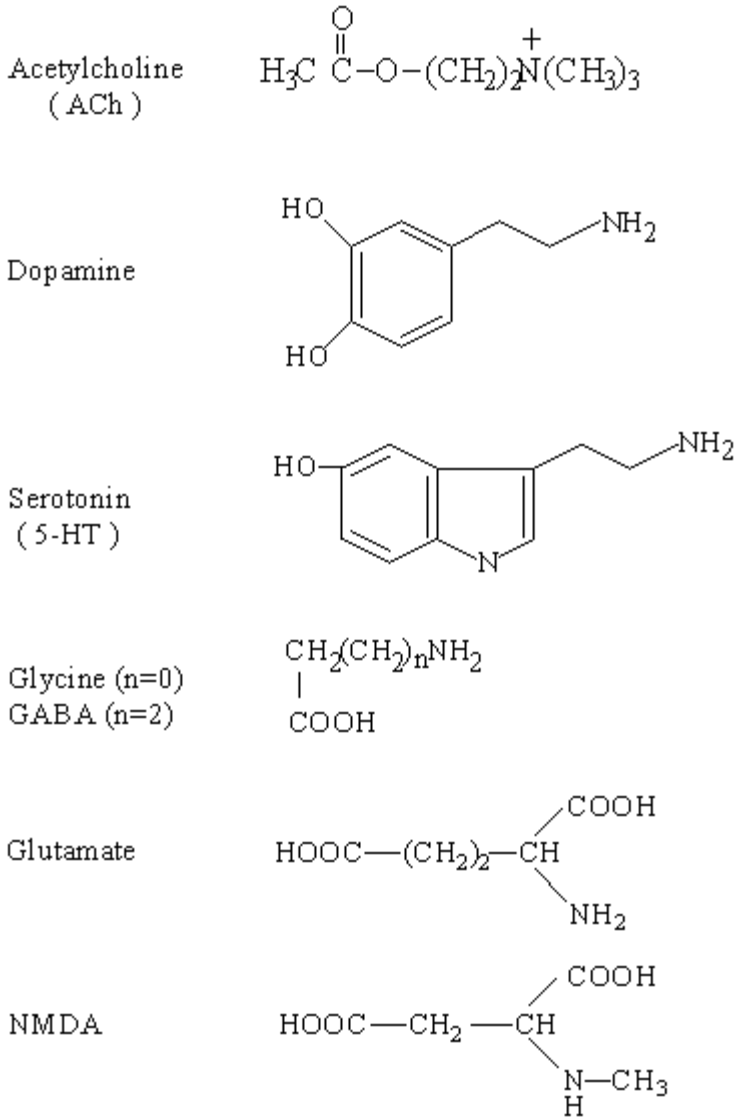

Figure 4.2. Chemical structures of major neurotransmitters.

The receptors for neurotransmitters can be classified into two categories: G-protein-coupled receptors and ionotropic receptors. G-protein-coupled receptors are involved in signal transduction. Like other agonists, binding of neurotransmitters on G-protein-coupled receptors may trigger a series of signaling processes (more info). The ionotropic receptors form an ion channel which may be activated upon binding of specific neurotransmitters. Upon activation, some

synaptic channels (e.g., acetylcholine and glutamate receptor channels) cause the postsynaptic membrane to depolarize while others (e.g., GABA and glycine receptor channels) cause hyperpolarization.

In the postsynaptic neuron, the membrane potential change at spines will propagate along the membrane to other regions, with possibility of being attenuated. Since a neuron contains many spines and each spine may cause membrane potential change, the membrane potential at any region is determined by the summation of these membrane potential changes when they reach the region. If the membrane is depolarized to a value above the threshold, the action potential will be generated at the region. If an action potential is generated at the "axon hillock" (the region connecting the cell body to the axon), it will be able to travel along the axon to the terminals.

Hyperpolarization has a negative effect on the generation of action potentials. For this reason, the depolarization potential at spines is often called "excitatory postsynaptic potential" (EPSP) and the hyperpolarization potential is called "inhibitory postsynaptic potential" (IPSP).

The glutamate receptor (GluR) channel plays a critical role in the Hebbian type of learning. It may be divided into three subtypes: NMDA, kainate and AMPA. All glutamate receptors bind efficiently to the glutamate molecule, but their binding affinity for other agonists varies. The NMDA subtype has a high binding affinity for the NMDA molecule, but low affinity for the kainate or AMPA molecule. Therefore, a channel formed by NMDA receptors can be activated by NMDA or glutamate, but not by kainate or AMPA. Similarly, the channel formed by AMPA receptors can be activated by AMPA or glutamate, but not by NMDA or kainate.

5. Learning - Overview

Learning, as recognized by Ivan Pavlov, is basically a process which associates two events. After we see rain following dark cloud a number of times, the dark cloud will very likely trigger the memory of rain before it actually appears. The association between rain and dark cloud allows us to avoid unpleasant events well ahead. This ability is important for survival. When one learns to play chess, he should first memorize the patterns which will immediately lead to the capture of the king. Let us call these patterns {A}. After a few weeks of experience (learning), he may find that some patterns, called {B}, could lead to {A}. The association between {A} and {B} also allows the player to better plan his next move before too late. After years of experiences, he may discover that some patterns {D} are likely to result in patterns {C}, and {C} may lead to {B}. Therefore, a novice player can see only a couple of steps ahead, but an experienced player can see many steps in advance.

A major goal of physical sciences is to find the equations associating the state of a system at a given time t and its next moment, $t + dt$. This enables physicists to predict future time courses, given the present information (an initial condition). For instance, meteorologists have been able to develop a set of equations describing the time courses of atmospheric conditions (temperature, pressure, etc.). From the present information provided by satellites, they can predict rain several days before the dark cloud is formed.

In our brain, the *hippocampus* (Fig. 5.1) is the major area for learning. In 1950s, a few patients were treated for epilepsy by removing part of the hippocampus. After the surgery, the patients still remembered their names, the events of their childhood, and the language learned before the surgery. However, they could not form new memory. Although they could talk normally with their relatives and friends, but they forgot such events in the next day. The early stage of Alzheimer's disease is characterized by the same symptom, because the hippocampus is usually the first area to be damaged in Alzheimer's patients.

At the cellular and molecular level, there are two distinct learning mechanisms: the Pavlovian type and Hebbian type. In the Hebbian type of learning, both presynaptic and postsynaptic neurons are involved. The Pavlovian type does not involve postsynaptic neurons. Learning in the hippocampus is mainly the Hebbian type.

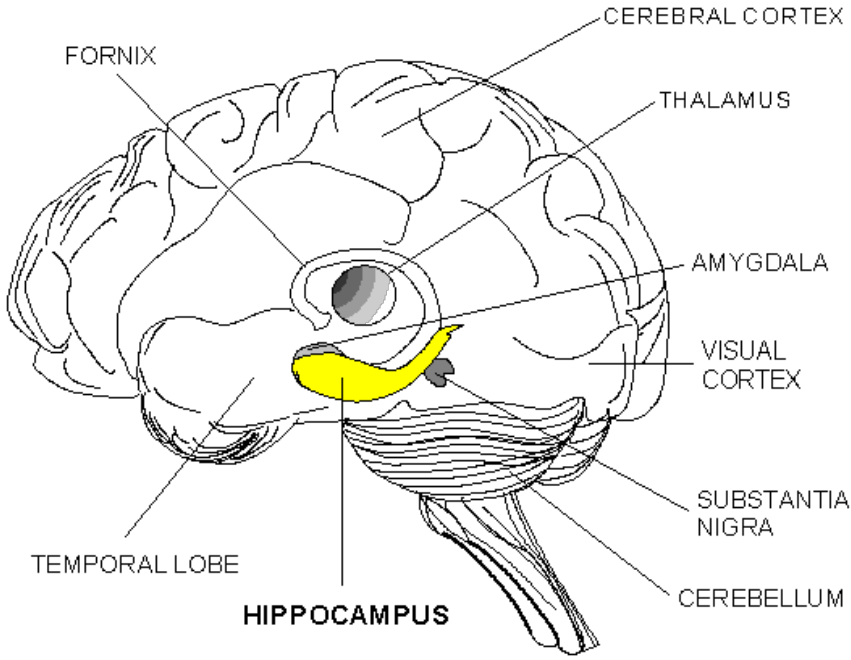

Figure 5.1. Schematic drawing of a human brain. The hippocampus is a major area for learning.

6. Pavlovian Type of Learning

The Pavlovian conditioning is an implicit learning which does not require consciousness of the subject. Hippocampus-impaired patients can still learn new motor skills through this mechanism. Even invertebrate animals with simple neural circuits may be trained by the Pavlovian conditional reflex, which is essentially the association between conditioned and unconditioned stimuli. In the original Pavlov's experiments on the dog, food is the unconditioned stimulus which elicits the secretion of saliva by native response. The unconditioned stimulus was preceded by a conditioned stimulus (the bell-ringing) which initially did not produce salivation. After a few paired stimulations, the bell-ringing itself could produce salivation.

The molecular mechanism of the Pavlovian conditioning has been well understood for the Aplysia system which contains a simple neural circuit. In this system, the unconditioned stimulus is a strong shock to the tail, eliciting gill movement by native reaction. The conditioned stimulus is a weak tactile stimulus to either siphon or mantle shelf, which initially does not cause the gill movement (Figure 6.1A). After a few paired stimulations (weak stimulus to siphon followed by tail shock), the weak stimulus to siphon alone can cause the gill movement. To explain its molecular mechanism, let us first explain the mechanism of **sensitization**, which refers to the potentiation of synaptic transmission by the unconditioned stimulus alone.

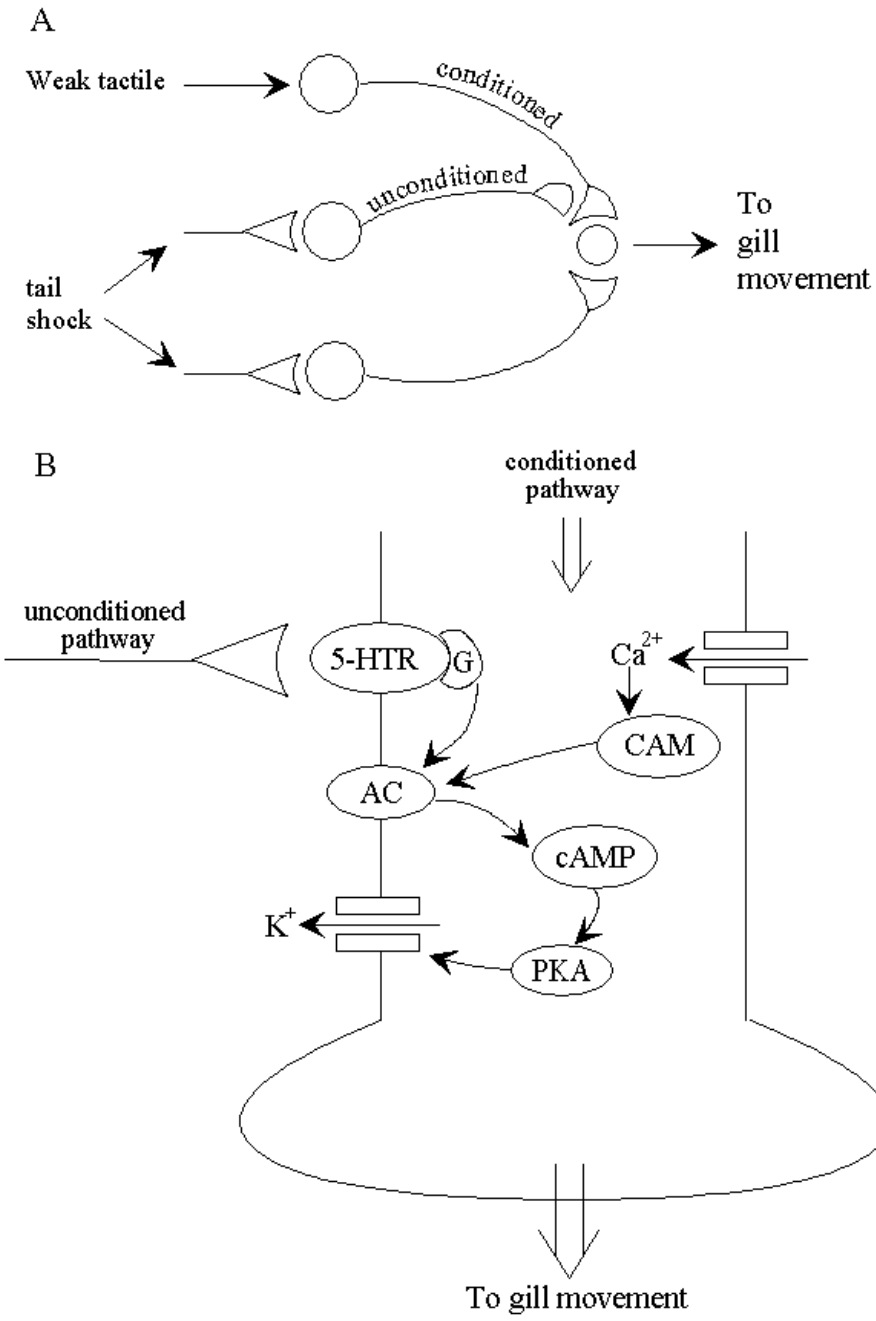

Figure 6.1. Molecular mechanism of the Pavlovian conditioning for the Aplysia system.

In Fig. 6.1B, the unconditioned stimulus (tail shock) not only elicits gill movement directly, but also activates a modulatory neuron which

releases 5-HT (serotonin) to bind on its G-protein-coupled receptor (5-HTR) in the nerve terminal of the conditioned pathway. This nerve terminal may release neurotransmitters to excite a postsynaptic neuron which is directly responsible for the gill movement. Binding of 5-HT to its receptor can activate adenylyl cyclase (AC), producing cAMP. The cAMP triggers phosphorylation of potassium channels by activating the protein kinase A (PKA). Phosphorylation of potassium channels in this system reduces channel opening probability. The reduction of K^+ currents slows the repolarization of action potentials, thereby increasing Ca^{2+} influx to release more excitatory neurotransmitters. Hence, the synaptic transmission in the conditioned pathway is potentiated.

The sensitization by the unconditioned stimulus alone may or may not be significant, depending on the system. As explained below, the efficacy of synaptic transmission in the conditioned pathway will be greatly enhanced if the unconditioned stimulus is paired with the conditioned stimulus.

In the conditioned pathway, Ca^{2+} ions may move into the nerve terminal upon the arrival of the action potential. Initially, the duration of the action potential is short so that the Ca^{2+} influx cannot release sufficient neurotransmitters to elicit the gill movement. However, after Ca^{2+} ions move into the nerve terminal, they may bind with calmodulin (CAM). The Ca^{2+}/calmodulin complex could also activate adenylyl cyclase to produce more cAMP for the phosphorylation of potassium channels. Therefore, the conditioned stimulus, if paired with the unconditioned stimulus, can amplify the activation of adenylyl cyclase. After a few paired stimulations, the reduction of K^+ outflux may increase the duration of action potentials to the extent that the conditioned stimulus (i.e., the weak stimulus to siphon) alone can induce sufficient Ca^{2+} influx to elicit the gill movement.

In the Pavlovian conditioning, the unconditioned stimulus has to be applied shortly after the conditioned stimulus. The optimal interval between the two stimuli was found to be 0.5 second for the case of Aplysia. The 0.5 second interval may correspond to the time required for the binding of Ca^{2+} with calmodulin. Thus, the adenylyl cyclase can be activated almost simultaneously by the Ca^{2+}/calmodulin complex from the conditioned pathway and the G protein from the unconditioned pathway.

Pavlovian Conditioning of Hermissenda: Current Cellular, Molecular, and Circuit Perspectives - Learning and Memory, 2004.

7. Hebbian Type of Learning

As discussed in the previous chapter, the Pavlovian type of learning involves only the presynaptic neuron. In the Hebbian type of learning, the synaptic modification is induced by the participation of both presynaptic and postsynaptic neurons.

Recalling that learning is a process that associates two events. In the hippocampus, events are represented by a population of neurons, each may be either excited or in the resting state. A particular event is represented by a particular set of neurons in the excited state. For instance, if the number of neurons involved in the representation is *n*, then mathematically an event can be denoted by a vector with the dimension *n*,

$$X = [x_1, x_2, x_3, ...x_n]$$

where x_i (i = 1 - *n*) is either "0" (resting) or "1" (excited).

The hippocampus contains the most complex neural network. Each neuron is connected to thousands of other neurons. For simplicity, we assume that only five neurons are involved in the representation of events. The connection of these neurons are shown in Figure 7.1. The lines are drawn from the nerve terminals (presynaptic) in the upper row to the dendrites (postsynaptic) in the lower row. For example, in Figure 7.1, the terminals of the first neuron are connected to the dendrites of the second and fourth neurons. The terminals of the second neuron are connected to the dendrites of the first and third neurons. These lines may also be considered as the synapses between neurons. The "dark cloud" is represented by the excitation of the second and fourth neurons and the "rain" is represented by the excitation of the first, fourth and fifth neurons.

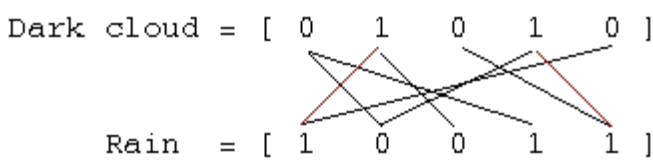

Figure 7.1. Illustration of the Hebbian type associative learning.

In the Hebbian type of learning, the synaptic modification may be induced only when both presynaptic and postsynaptic neurons are excited. In Figure 7.1, these synapses are represented by red lines. The left red line connects the second and first neurons; the right red line connects the fourth and fifth neurons. When the dark cloud and rain happen almost at the same time, these neurons are excited and their synapses are modified so that nerve impulses can be more easily transmitted from the presynaptic neuron to the postsynaptic neuron. Suppose before learning the nerve impulse is unlikely to transmit from the second neuron to the first neuron. After the pairing between dark cloud and rain, this synaptic transmission is greatly enhanced. Next time, the dark cloud alone is likely to cause the excitation of the first neuron, thereby increasing the probability to recall rain.

How could physiological system implement Hebbian type of learning? The answer lies in the NMDA channel, which is a subtype of glutamate receptor channels. For most synaptic channels, activation (opening) requires only the binding of neurotransmitters. However, activation of NMDA channels requires two events: binding of glutamate (a neurotransmitter) and relief of Mg^{2+} block. NMDA channels are located at the postsynaptic membrane. When the membrane potential is at rest, the NMDA channels are blocked by Mg^{2+} ions. If the membrane potential is depolarized due to excitation of the postsynaptic neuron, the outward depolarizing field may repel Mg^{2+} out of the channel pore. On the other hand, binding of glutamate may open the gate of NMDA channels (its mechanism is not known). In the normal physiological process, glutamate is released from the presynaptic terminal when the presynaptic neuron is excited. Relief of Mg^{2+} block is due to excitation of the postsynaptic neuron. Therefore, excitation of both presynaptic and postsynaptic neurons is necessary and sufficient to open NMDA channels.

Another important feature of the NMDA channel is that it conducts mainly the Ca^{2+} ion which may activate various enzymes for synaptic modification. The enhancement of synaptic transmission is called long-term potentiation (LTP), which involves two parts: **induction** and **maintenance**. The induction refers to the process which opens NMDA channels for the entry of Ca^{2+} ions into the postsynaptic neuron. The subsequent synaptic modification by Ca^{2+} ions is referred to as the maintenance of LTP. We have just explained the mechanism for the induction of LTP. The maintenance of LTP is accomplished by insertion of ionotropic receptors (e.g., AMPA receptors) into

postsynaptic membranes.

AMPA receptors are a subtype of glutamate receptors. They may form ion channels in the postsynaptic membrane to conduct small cations. Higher density will generate greater ionic influx when glutamate molecules are released from the presynaptic terminal, thereby resulting in more membrane depolarization, which in turn facilitates excitation of the postsynaptic neuron. It has been shown that Ca^{2+} can activate calcium/calmodulin-dependent protein kinase II (CaMKII) and drive AMPA receptors into postsynaptic membranes (reference).

The insertion of AMPA receptors into postsynaptic membranes is a form of short-term memory. It cannot last very long because AMPA receptors are constantly cycled into and out from postsynaptic membranes due to a constitutive pathway and busy activities at the hippocampus (review 1; review 2). For long-term storage of memory, the short-term memory must be converted into long-term memory. Its mechanisms are discussed in the next few chapters.

8. Microtubules and Memory Formation

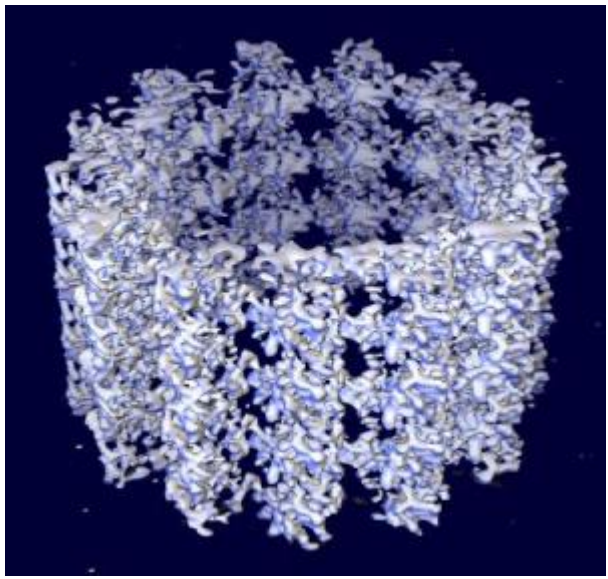

Figure 8.1. A microtubule segment from cryo-electron microscopy. [Credit: U.S. Department of Energy].

Microtubules are a major component of cytoskeleton responsible for intracellular transport of various cargos such as vesicles, organelles and neurotransmitter receptors. In Chapter 7, we have seen that the short-term memory is formed by inserting neurotransmitter receptors (e.g., AMPA receptors) into postsynaptic membranes at dendritic spines. However, the ultimate source of neurotransmitter receptors is protein synthesis at the cell body. Transport of neurotransmitter receptors from the cell body to spines requires microtubules (reference). Therefore, microtubules could be involved in memory formation and storage. This idea is supported by the following evidence:

1. Alzheimer's disease (loss of memory) is characterized by neurofibrillary tangles, resulting from damages to microtubules.
2. Microtubule-depolymerizing agents cause amnesia (reference).
3. Microtubules can enter dendritic spines and modulate spine morphology (reference 1; reference 2).
4. Many microtubules move from dendritic shafts to spines in hippocampal CA1 neurons after tetanic stimulation during long-term potentiation (reference).

A microtubule is a hollow cylinder with diameter about 25 nanometers. It consists of 13 protofilaments. Each protofilament is composed of tubulin dimers, with the α subunit of one dimer attached to the β subunit of the next dimer. Thus, a protofilament has one end (called "minus" end) with the α subunit exposed and another end (called "plus" end) with the β subunit exposed. Note that the definition of "+" and "-" on both ends does not imply that protofilaments are permanent dipoles. Under physiological conditions, protofilaments and the entire microtubule are highly negatively charged because the C-termini of both α and β subunits are rich in acidic (aspartate and glutamate) residues (reference).

Elongation of a microtubule is usually from the plus end. In the absence of microtubule-associated proteins (MAPs), a microtubule may shrink. MAPs also regulate the connection between a microtubule and neighboring microtubules or other cellular structures. In general, binding of MAPs to a microtubule makes it more stable and rigid. Their binding is determined by MAP phosphorylation, which in turn may be regulated by calcium ions (see Molecular Biology of the Cell). The tau protein involved in Alzheimer's disease belongs to MAPs. Its malfunction is due to hyperphosphorylation (review).

Kinesin and dynein are the major motor proteins mediating microtubular transport. These motor proteins can carry cargos and "walk" along a microtubule. Kinesin walks toward the plus end, but dynein walks toward the minus end (review 1; review 2). Neurotransmitter receptors are predominantly carried by kinesin which walks toward the plus end. As we shall see in the next chapter, the polarity and specific directional transport make microtubules a good binary system for memory encoding and storage.

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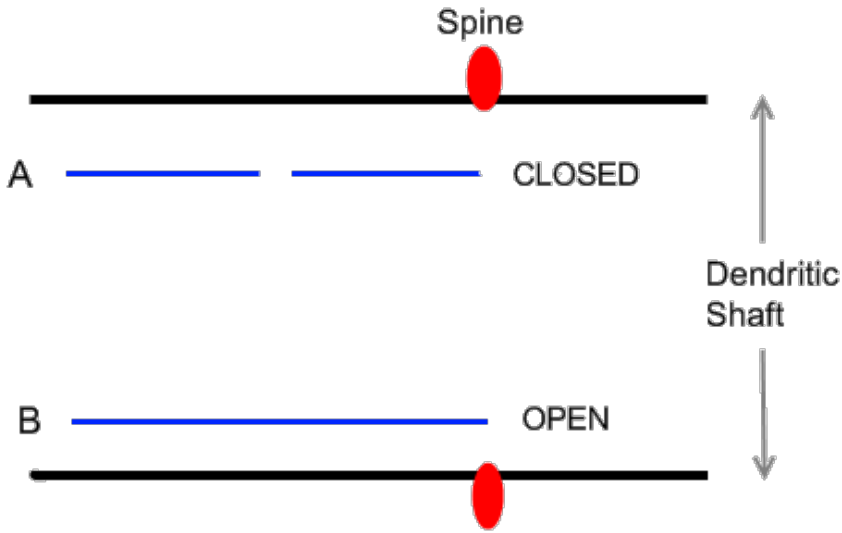

Figure 9.1. A model for memory encoding in the microtubular network. A blue line represents a microtubule molecule. (A) The "CLOSED" state. A microtubular track is interrupted before reaching its destination (e.g., a spine). Transport along this track is inefficient. (B) The "OPEN" state. A microtubular track is continuous up to its destination.

It has been observed that, for vertebrate neurons, microtubules in axons are unidirectional, with their plus ends distal to the cell body (plus-end-out). In dendrites, however, their polarity is mixed (reference 1; reference 2), and age-dependent (reference).

The microtubular polarity in axons can easily be understood. Most neurotransmitter receptors are produced at the cell body while their destination in an axon is its terminal. Transport of neurotransmitter receptors by microtubules is predominantly mediated by the motor protein kinesin (reference), which walks toward the plus end. This explains why the microtubular polarity in axons should be plus-end-out.

In dendrites, the destination of neurotransmitter receptors is spines (small membranous protrusions which form synapses with axon terminals), located away from the cell body. For kinesin to carry

neurotransmitter receptors from the cell body to spines, the microtubular polarity in dendrites should also be plus-end-out. Then, why do dendrites need minus-end-out microtubules? A possible answer lies in another motor protein dynein, which walks toward the minus end. Dynein is responsible for the retrograde transport of membranous organelles from the axon terminal to the cell body. It can also mediate the transport of endosomes and the Golgi complex (review). Recently, dynein has been demonstrated to play a crucial role in dendritic branching (reference). This result is consistent with another finding that, in Drosophila (fly) dendrites, microtubular orientations are predominantly minus-end-out. These minus-end-out microtubules are used by dynein for dendritic growth (reference).

Although memory appears to be stored in synapses, it is the microtubular network which is calling the shots. Without dynein, dendrites cannot grow or branch out. Without kinesin, no neurotransmitter receptors are available for modifying synaptic strength. The two motor proteins walk along microtubular tracks. Therefore, memory could be encoded in the opening or closing of these microtubular tracks.

In axons, microtubules are continuous from the cell body to the axon terminal, but in dendrites they are interrupted. Figure 9.1 presents a simple binary model which may be used for memory encoding and storage. A microtubular track to its destination (e.g., a spine) is "OPEN" if a microtubule is continuous up to the destination. If a track is interrupted, namely, consisting of two or morel shorter microtubules, then the track is "CLOSED" because transport along this track will be inefficient.

This model is based on the observation that, in apical dendrites of mice, the average microtubular length increases with age (reference). It seems that experience can leave two types of memory traces: (1) insertion of neurotransmitter receptors into postsynaptic membranes (see Chapter 7) and (2) modification of microtubular tracks. The length of a microtubule is directly controlled by microtubule-associated proteins (MAPs) which, in turn, is regulated by calcium ions. Memory traces generally increase the length of microtubules so that motor proteins can walk continuously toward their destination.

In addition to their effect on the length of microtubules, memory traces are expected to make microtubules more rigid as MAPs may fix microtubular tracks to specific destinations. The next chapter will provide further evidence that the portion of short and free microtubules declines with age.

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Types of Memory Storage

It has been well established that experience can leave memory traces by inserting neurotransmitter receptors into postsynaptic membranes. This type of memory traces are usually short (minutes to hours), because neurotransmitter receptors are constantly cycled into and out from postsynaptic membranes due to a constitutive pathway and busy activities at the hippocampus (review 1; review 2). Since microtubules are essential for transporting neurotransmitter receptors and other molecules for synaptic strength and dendritic growth, Chapter 9 proposed another type of memory traces: modification of the microtubular network. Such memory traces require not only calcium ions, but also microtubule-associated proteins (MAPs). They are more difficult to create and thus harder to be replaced by new events. Its memory capacity, however, is limited by the number of microtubules. The hippocampus, which is the first region to acquire memory, has a small size. Old memory could be replaced by new memory within days. For long term storage, the memory stored in the hippocampus must be transferred to other brain regions such as the neocortex (review). There is strong evidence suggesting that the hippocampus and neocortex communicate with each other for memory consolidation (reference 1; reference 2).

Memory Consolidation During Sleep

Electroencephalogram (EEG) exhibits several types of brain waves. The one with the lowest frequency (~1 Hz) is called the delta wave, which is predominant in babies and also observed in children and adults during **slow wave sleep** - the sleep characterized by the slow delta wave. Slow waves have been demonstrated to play a critical role in memory consolidation (review). They becomes less likely to occur as one ages (reference 1; reference 2).

A slow wave oscillates between a depolarization phase and a hyperpolarization phase, each lasting for several hundred milliseconds. The hyperpolarization phase is known as the "DOWN state", during which nearly all cortical neurons are silent. The depolarization phase is called the "UP state" with intense firings by cortical neurons. The slow wave can originate from anywhere in the

cortex spontaneously (reference), and propagate to other regions as a traveling wave (reference). It has been well established that the slow wave is coupled to "sharp waves" which originate in the hippocampus. UP states have been found to precede hippocampal sharp waves (review). These results indicate that, during slow wave sleep, the neocortex sends message to the hippocampus, looking for new memory traces. The hippocampus replies by generating sharp waves, which then travel to the neocortex and modify its memory storage.

A related feature of brain waves is the K-complex, which consists of a large amplitude biphasic fluctuation, either from "down" to "up" or from "up" to "down" (reference). The duration of the K-complex is about the same as a single cycle (~1 second) of the slow wave. K-complexes are also less likely to occur as one gets older.

Mechanisms of Slow Waves

The spontaneous generation of UP states could arise from excitation by glutamates (reference), but the mechanism for DOWN states is less clear. It has been proposed that the hyperpolarization was caused by outward K^+ currents, possibly through ATP-sensitive K^+ channels (reference), because the intense firing during the UP state may increase the intracellular Na^+ level, which then activates Na^+/K^+-ATPase and consumes ATP. The ATP-sensitive K^+ channels open when the ATP level decreases. This model sounds reasonable. However, it depends on preceding intense firings while the K-complex may not have a preceding up phase (reference). It is also unclear how the model can explain the age-dependence of both slow waves and K-complexes.

Here enters the microtubule. As mentioned in Chapter 9, the average length of microtubules in dendrites is age-dependent. Shorter microtubules may represent those which are not fixed in a microtubular track toward specific destination. If somehow these free microtubules can cause the DOWN state, then the age-dependence of both slow waves and K-complexes can be explained. The question is: how?

Microtubules are highly negatively charged, about 50 electrons per tubulin dimer (reference). If they can adsorb on the membrane surface, they will have significant effects on membrane properties. Strictly speaking, the opening probability of voltage-gated ion channels depends not only on the membrane potential, but also on the local electric field from nearby surface charges. For instance, high

extracellular Ca^{2+} concentration, which neutralizes the negative surface charges of a cell membrane, can shift the voltage dependence of Na^{+} and K^{+} channels (those involved in the generation of action potentials) by as much as 30 mV (reference). Since surface charges are usually fixed, their effects can be ignored in most cases. However, in our case, the surface charges play a crucial role.

Cellular membrane surfaces are negatively charged. To adsorb on the membrane surface, microtubules must rely on counterions, which can mediate attraction between like-charges (reference 1; reference 2). Multivalent counterions are usually more effective than monovalent counterions. In the following, we shall show that the DOWN state can be explained by the assumption that Ca^{2+} ions, but not monovalent cations, can mediate the adsorption of microtubules on the intracellular membrane surface.

Synaptic stimulation may cause Ca^{2+} accumulation near the postsynaptic site. Part of them may loosely bind to the negative charges on the intracellular membrane surface, forming a layer of counterions. These Ca^{2+} ions make the nearby ion channels feel more depolarized than the membrane potential. Thus, binding of Ca^{2+} ions on the intracellular membrane surface makes a neuron more excitable. On the other hand, these Ca^{2+} ions can mediate the adsorption of free microtubules if they are around. As a result, the Ca^{2+} ions serve as the counterions for both microtubules and membrane surface charges. The adsorption of highly negatively charged microtubules should make nearby ion channels feel more hyperpolarized, which counteracts the excitatory effects of Ca^{2+} ions. If sufficient number of free microtubules can adsorb on the membrane surface, neuronal firings will be suppressed, resulting in the DOWN state. Since memory traces generally fix microtubules in a specific track toward its destination, the number of free microtubules should decrease with age. This explains the age-dependence of the DOWN state.

This model is supported by the observed duration of Ca^{2+} accumulation. In the absence of additional synaptic stimulation, Ca^{2+} ions eventually diffuse away. The adsorbed microtubules should also leave the membrane surface because they cannot bind to the membrane surface without divalent counterions. In basal dendrites, upon stimulation by glutamate microiontophoresis, the duration of Ca^{2+} accumulation was found to be about 1 second (reference, figure 1) - the same as a single cycle of the slow wave. This experiment also simultaneously measured the membrane potential at the soma. The duration of the somatic plateau depolarization was about 300 milliseconds. This means that Ca^{2+} accumulation can last much

longer than the somatic plateau depolarization. According to the present model, the period during the somatic plateau depolarization may be identified as the UP state, and the subsequent period until substantial decrease in Ca^{2+} accumulation could be the DOWN state, during which sufficient Ca^{2+} accumulation can mediate the adsorption of free microtubules to suppress neuronal firings.

The present model requires Ca^{2+} accumulation to induce the DOWN state, but it does not require preceding intense firings. As mentioned above, some K-complexes do not have a preceding up phase. These K-complexes may originate from synaptic stimulation which causes Ca^{2+} accumulation, but without generating action potentials.

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